Mutagenesis protocol for site direct mutagenesis

Introduction
This protocol in based in a routine Phusion® PCR by New England BioLabs
These guidelines cover a site direct mutagenesis.

Reaction Setup: We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (98°C). All components should be mixed and centrifuged prior to use. It is important to add Phusion DNA Polymerase last in order to prevent any primer degradation caused by the 3´→ 5´ exonuclease activity.

PCR recipe

Component	**20 µl Reaction**	**50 µl Reaction**	**Final Concentration**
Nuclease-free water	to 20 µl	to 50 µl	
5X Phusion HF or GC Buffer	4 µl	10 µl	1X
10 mM dNTPs	0.4 µl	1 µl	200 µM
10 µM Forward Primer	0.2µl	0.5 µl	0.25 µM
10 µM Reverse Primer	0.2µl	0.5 µl	0.25 µM
Template DNA	100-500ng	100-500ng	variable
DMSO (optional)	(0.6 µl)	(1.5 µl)	3%
Phusion DNA Polymerase	0.2 µl	0.5 µl	1.0 units/50 µl PCR

Transfer PCR tubes from ice to a PCR machine with the block preheated to 98°C and begin thermocycling

PCR Program

STEP	**TEMP**	**TIME**
Initial Denaturation	98°C	30 seconds
	98°C	5-10 seconds

16-20 Cycles	55°C 72°C	10-30 seconds 15-30 seconds per kb
Final Extension	72°C	5-10 minutes
Hold	37°C*	1-2 Hrs**

*Set storage temperature at 37°C, another option is place the cooled tube inside a 37°C incubator. **Once that the program is complete with the two primers, in the holding step add 1 μl DpnI restriction enzyme to the PCR tube directly. Only digestiong the PCR products for no more that 2 hrs. (Purification is not necessary at this stage).

Transform purified DNA into highly competent cells.

Screen the transformants for the desired mutation using colony PCR, restriction digest or sequencing as appropriate.

Extra
Primers phosphorylation of 5'

1. Mix:
2. 3μ 100μM sense oligo (final concentration 10μM)
3. 3μ 100μM anti-sense oligo (final concentration 10μM)
4. 3μ 10X T4 DNA ligase buffer
5. 2μ T4 Polynucleotide kinase (PNK)
6. 19μ double distilled water
   (Total volume 30 μL)

7. Incubate at 37^{o}C for 1.5Hrs
8. Heat kill PNK by 10 min at 70^{o}C